NATURAL HYBRIDIZATION OF HELIANTHUS LONGIFOLIUS WITH H. ATRORUBENS AND H. OCCIDENTALIS[1]

DALE M. SMITH AND WILLIAM C. MARTIN

Several studies of natural hybridization between species of *Helianthus* have been made in recent years. Many natural hybrids have been reported and most of them have been substantiated by controlled crossing. In general, whenever two diploid species grow in close proximity under disturbed conditions, local hybrid swarms result. The gene flow may be restricted as in the case of *Helianthus divaricatus* × *H. microcephalus* (Smith and Guard, 1958), or extensive introgression may occur as in *H. annus* × *H. bolanderi* (Heiser, 1949). Regardless of the extent of hybridization, all of the previously described examples have dealt with pairs of species.

In September of 1957, a mixed population consisting of four species of *Helianthus* was found one mile northeast of Albertville, Alabama. These species were *H. microcephalus, H. longifolius, H. occidentalis,* and *H. atrorubens.* Putative hybrids of *H. longifolius* × *H. atrorubens,* and *H. longifolius* × *H. occidentalis* were discovered, but *H. microcephalus* apparently had not hybridized with the other species. The area in which the mixed population was found was a relatively new highway embankment bordering a small scrub-oak woodlot. The four species were more or less aggregated at specific places within the area, and hybrids were found only at the zones where two species overlapped. At the west end of the population there were a few scattered individuals of *H. occidentalis,* and approximately 200 yards distant at the east end of the area was a large population of *H. atrorubens* with scattered individuals of *H. longifolius* between these first two species. *Helianthus microcephalus* was found at the edge of the oak woodlot adjacent to the plants of *H. atrorubens* and *H. longifolius.* While mixed populations of *H. atrorubens* and *H. microcephalus* are quite commonly en-

[1] Supported by the University of Kentucky Faculty Research Fund and *NSF* grant #G-1836. The authors express their appreciation to Dr. C. B. Heiser, Department of Botany, Indiana University, for valuable suggestions following a critical appraisal of the manuscript.

countered in many places, the other two species are rarely ever seen in the southeast much less being found in mixed populations. *Helianthus occidentalis* is a common plant of the prairies and sand hills of the midwest from Michigan to Texas, and is occasionally encountered in the southeast. *Helianthus longifolius* is one of the rarest species of *Helianthus,* and its range covers only a small area in the northern parts of Alabama and Georgia, where it typically grows on or near rock outcroppings. *Helianthus atrorubens* and *H. microcephalus* are widely distributed throughout the southeast in a variety of habitats. The reasons for this unusual occurrence of four species in such a limited area are somewhat obscure at the moment, but it seems possible that the plants could have been introduced into the area along with fill material which was used in constructing the embankment, since the plants are perennials and their underground parts could be transported along with soil very easily.

Procedures

Herbarium specimens and living plants, as well as flower buds for cytological examination were taken from the population. Selected individuals and a random mass collection were pressed for later study. Whole plants were pressed, since the basal material as well as the upper parts of the plants possessed important morphological features for comparison. Herbarium specimens have been deposited at Indiana University and the University of Kentucky. The cytological material was fixed in a mixture of 3 parts ethyl alcohol to 1 part acetic acid for 24 hours, and transferred to 70% ethyl alcohol for storage. Anthers of the appropriate age were squashed in acetocarmine for study.

Morphology

The species occurring in this population are quite distinct and are not easily confused, although *H. atrorubens, H. occidentalis,* and *H. longifolius* all have the upper cauline leaves reduced, giving the plants a decidedly scapose aspect. The leaves of *H. microcephalus* are essentially similar along the length of the stem except in the inflorescence where they are reduced. The leaves and stems of *H. longifolius* are completely glabrous, so

that it too is quite distinct. Actually, only *Helianthus occidentalis* and *H. atrorubens* present a somewhat similar aspect, but the purple disk corollas and obtuse phyllaries of *H. atrorubens* serve to distinguish it quite adequately. The outstanding morphological features of the four species are presented in Table 1. The morphology of the putative hybrids will be considered individually.

Helianthus longifolius × H. occidentalis

One putative hybrid of this combination was discovered and appeared to be essentially intermediate between the two parents. Especially evident were the modifications of leaf shape and pubescence, the hybrid having narrowly lanceolate leaves which were setaceous on the margin.

Helianthus longifolius × H. atrorubens

The many usually well defined differences between these two species are, in this population, bridged by hybrid individuals. Considerable variation was encountered in leaf width, phyllary shape, head diameter, disk color and leaf pubescence, which were analyzed in this study, and are summarized in Table 2. The lower stature and more prominent basal rosettes of *H. longifolius* are usually sufficient to separate it from *H. atrorubens* but were not used here because of the great environmental modification of these two characteristics in this population.

LEAF SHAPE. The basal leaves in *H. longifolius* are generally numerous, elongate and linear or narrowly obovate, rarely exceeding one centimeter in width. In *H. atrorubens*, the leaves show considerable variability in shape, but are always many times broader than one centimeter. In this population the hybrids bridge the gap between these two extremes.

LEAF PUBESCENCE. Trichomes are completely lacking in *H. longifolius* except for a few at the tips of the receptacular bracts. In contrast to this, *H. atrorubens* is usually rather harshly pubescent over most of the plant. The leaves of the putative hybrids are characterized by the presence of a few scattered trichomes.

PHYLLARY SHAPE. One of the most outstanding features of *H. atrorubens* is the presence of broad, obtuse phyllaries. Those of

TABLE 1

Comparison of Morphological Features of *Helianthus atrorubens, H. occidentalis, H. longifolius, and H. microcephalus*

	H. atrorubens	*H. occidentalis*	*H. longifolius*	*H. microcephalus*
STEM PUBESCENCE	Spreading-hirsute	Strigose	Glabrous	Glabrous
LEAF				
Arrangement	Largely basal	Largely basal	Largely basal	Distributed along stem
Shape	Broadly ovate, narrowed to broadly winged petiole	Ovate, narrowed to slightly winged petiole	Linear to linear-lanceolate, gradually tapering to base	Ovate-lanceolate with attenuate tip. petiole distinct
Pubescence	Scabrous to hirsute	Scabrous to strigose	Glabrous	Scabrous above, tomentulose beneath
Width	3.5-5.5 cm.	2.5-4.0 cm.	0.9-1.5 cm.	2.5-5.0 cm.
PHYLLARY SHAPE	Short, broad, tips obtuse	Narrowly attenuate	Narrowly attenuate or acuminate	Narrowly attenuate or acuminate
DISK				
Diameter	1.2-1.5 cm.	0.8-1.3 cm.	0.9-1.3 cm.	0.5-1.0 cm.
Corolla color	Lobes purple	Lobes yellow	Lobes yellow	Lobes yellow

H. longifolius are linear attenuate while the hybrids' are merely acute.

DISK DIAMETER. The differences here are not pronounced but, in general, the disks of *H. longifolius* are about 1.0 centimeter in diameter whereas those of *H. atrorubens* range up to 1.5 centimeters.

DISK COLOR. A clear cut difference exists here in that *H. longifolius* has disk corollas which are yellow throughout, while *H. atrorubens* has purple-tipped corollas. In the field the putative hybrids appear to have yellow corollas but an examination under the microscope reveals that the hybrids have purple pigmentation on their tips. The pictorialized scatter diagram in figure 1 points out the discordant pattern of variation encountered in the population.

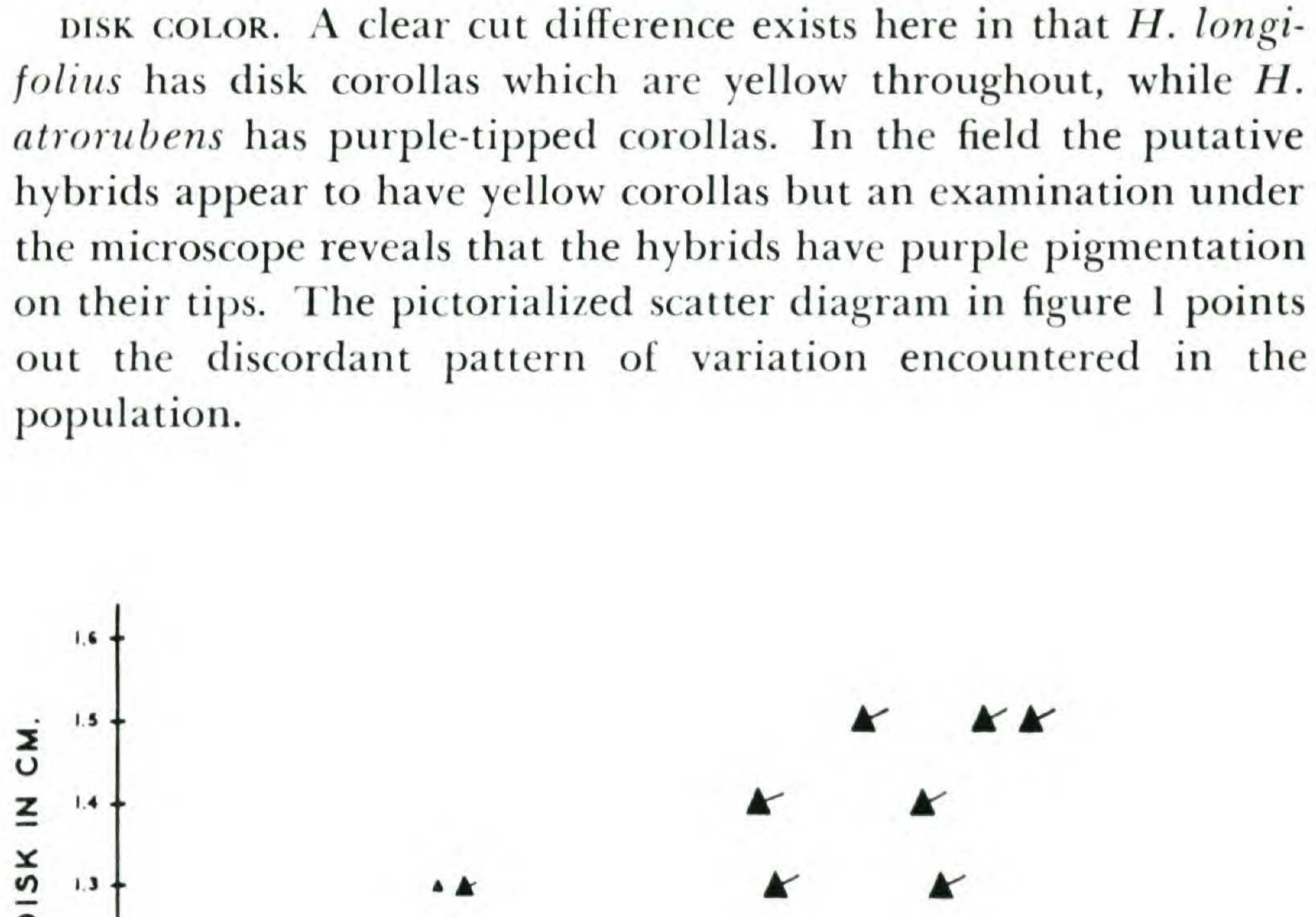

FIGURE 1. Pictorialized scatter diagram of a population sample including *Helianthus longifolius, H. atrorubens,* and putative hybrids. The smallest triangles = attenuate phyllaries, largest triangles obtuse phyllaries, intermediate triangles = acute phyllaries. Open triangles = yellow disk corollas, solid triangles = purple-tipped disk corollas. Triangles without a diagonal line on right side = glabrous leaves, with a long line = scabrous or hirsute leaves, with a short line = remotely setaceous leaves.

Cytology

The chromosome numbers of the species, with the exception of *H. longifolius,* which is reported here for the first time as N=17, have been presented by Heiser and Smith (1955), and are diploid (N=17). Meiosis in the parental species has been reported as normal in all except *H. atrorubens,* in which Jackson and Guard (1957) have reported numerous abnormalities. Plants of *H. atrorubens* which have been grown in the experimental garden have occasionally failed to produce pollen or viable seed, which may be an expression of an abnormal chromosomal condition. This is further suggested by the occurrence of low pollen fertilities in plants from the Alabama population which appear to be "good" *H. atrorubens.*

Table II

Comparison of Morphological Features of *Helianthus atrorubens,* *H. longifolius,* and their Putative Hybrid

	H. atrorubens	Intermediates	*H. longifolius*
LEAF			
Pubescence	Scabrous or hirsute	Remotely setaceous	Glabrous
Width	3.5-5.5 cm.	1.6-3.4 cm.	0.9-1.5 cm.
PHYLLARY SHAPE	Short, broad, tips obtuse	Short, broad, tips acute	Narrowly attenuate or acuminate
DISK			
Diameter	1.2-1.5 cm.	1.1-1.3 cm.	0.9-1.3 cm.
Corolla color	Lobes purple	Tips of lobes purple	Lobes yellow

However, in all the plants from this population which have been examined cytologically, parental species and putative hybrids, meiosis was essentially normal. Buds of several putative hybrids were examined, and of 65 microsporocytes examined, 63 showed perfect pairing and only 2 showed abnormal associations in the form of a single chain of 4 chromosomes.

Discussion

This report of natural hybridization in *Helianthus* is especially interesting in that four potentially hybridizing species are represented in a mixed population. Even though the evidence suggests that only three of the four species have actually hybridized, the

stage is nevertheless set for the formation of bizarre types of individuals incorporating genetic material from all four species. That such a phenomenon is theoretically possible, has been verified by three and four species hybrids which have been produced experimentally (Smith, unpublished). The actual discovery of hybrids of this type in nature would have special significance relative to the problems concerning the origin of polyploid species of *Helianthus*. There are several polyploids which show no close resemblance to extant diploid species or to the F_1 diploid interspecific hybrids which have thus far been produced. Consequently, it is suggested here that certain polyploid species may have originated from hybrids involving more than two species.

The relatively high fertility and the high degree of chromosome homology of the hybrids of *H. atrorubens* × *H. longifolius* serves to point up the lack of great barriers to crossing between the diploid perennial species of *Helianthus*, even in the presence of a high degree of morphological distinction. Furthermore, the very narrow distribution of *H. longifolius* contrasts sharply with the extensive range of *H. atrorubens*, and ecological differences probably also exist, so that the two species are not found in mixed populations unless they are brought together by disturbing influences. It would appear that genetic barriers have been of minor importance in speciation in this group; therefore, with a complete breakdown of the external barriers separating these species, it is conceivable that an amalgamation of them could occur. As yet, there is little evidence that this is happening even in such a disturbed area as that described here.

Summary

A mixed population of diploid (N=17) perennial sunflowers composed of *Helianthus atrorubens, H. occidentalis, H. longifolius* and *H. microcephalus* was found in September, 1957, near Albertville, Alabama. One putative hybrid between *H. occidentalis* and *H. longifolius* was found, and no hybrids involving *H. microcephalus* were evident but a large hybrid swarm of *H. atrorubens* × *H. longifolius* was present. These plants were quite variable, with leaf and phyllary characteristics showing

outstanding variation. Very little meiotic abnormality was encountered in the population, although the occurrence of low pollen fertilities in some of the plants suggests that cryptic structural differences in the chromosomes exist. Speciation has apparently proceeded without the development of the sterility barrier, but the external barriers which ordinarily separate them have in this instance been partially broken down by the influence of man. However, even under these conditions there is little indication of the amalgamation of the species in this population.

— UNIVERSITY OF KENTUCKY, LEXINGTON, AND UNIVERSITY OF NEW MEXICO, ALBUQUERQUE.

Literature Cited

Heiser, C. B., Jr. 1949. Study in the Evolution of the Sunflower Species *Helianthus annuus* and *H. Bolanderi*. Univ. Calif. Pub. Bot. **23:** 157–208.

Heiser, C. B. Jr. and D. M. Smith. 1955. New Chromosome Numbers in Helianthus and Related Genera (Compositae). Proc. Indiana Acad. Sci. **64:** 250–253.

Jackson, R. C. and A. T. Guard. 1957. Analysis of some Natural and Artificial Hybrids in Helianthus. Proc. Indiana Acad. Sci., **66:** 306–317.

Smith, D. M. and A. T. Guard. 1958. Hybridization between *Helianthus divaricatus* and *H. microcephalus*. Brittonia, **10:** 137–145.

Wallpaper Cleaner in the Herbarium. — In a well-kept herbarium, specimen sheets are handled in such a manner that they do not become excessively dirty. However, where collections have been neglected for long periods or are housed in poor cases they may become badly soiled by dust and smoke. This was the case with a rather large number of sheets in the Herbarium of Yale University, most of which had come as gifts and were dirty when received. Specimens of little value may be discarded but valuable collections should, of course, be preserved even though soiled. If the plant is not glued too tightly it may sometimes be removed and mounted on a clean sheet. However, where this is impossible without severe breakage some other method must be sought.